

Presence Of Multi Drug Resistant Coliform Bacteria Isolated From Biofilm Of Sachet And Borehole Waters Sold In Abakaliki Metropolis, Ebonyi State, Nigeria.

Okafor Collins Onyebuchi Okeke, Nwadiogbu Ifeanyi Anthony, Agumah Nnabuife Bernard, Eni Obehi Eugene, Odinkemere Stephen Chijindu

ABSTRACT: This study investigated the presence of multi drug resistant coliform bacteria from biofilm of sachet and borehole waters sold in Abakaliki metropolis in Ebonyi State, Nigeria. Five hundred (500) samples of water comprising 250 each from selected brand of sachet water retailers and borehole water dispensers from seven locations were sampled for the detection of coliform bacteria from biofilm and to determine their antimicrobial susceptibility using commercially prepared antibiotic discs. Results revealed a high faecal contamination level in sachet waters as Gospel 36 (72%), Aqua Rapha 30 (60%) and Bejoy 18 (36%) were the highest among the sachet water brands examined, with Nene and Rock Tama sachet water brands having the lowest contamination level of 6(12%) and 13(26%) respectively. Borehole samples, results revealed that Abofia had 27 (76.93%) samples contaminated with faecal bacteria while Azugwu 11 (28.5%), Azuiyokwu 18 (50%), Azuiyudene 29(80%), Kpirikpiri 24 (66.63%), Presco/Ntezi 16(46.15%) and Udensi 22 (61.54%). *Escherichia coli*, *Enterobacter* spp and *Klebsiella* spp were the major contaminants of both sachet and borehole water samples. The bacteria isolates from biofilm of sachet and borehole waters were susceptible to only three of the antibiotics used namely nitrofurantoin, amoxicillin and ampicillin. The bacteria were completely resistant to ciprofloxacin, tetracycline, norbactin/norfloxacin, ofloxacin, cefuroxime and gentamicin. This showed that they exhibit multi-drug resistance pattern which is a common feature of medically important biofilm bacteria. We therefore report the presence of multi-drug resistant coliform bacteria from biofilm of sachet and borehole waters sold in Abakaliki metropolis, Ebonyi State, Nigeria.

Key words: Biofilm, coliform, bacteria, multi-drug resistance, sachet, borehole, water.

INTRODUCTION

Biofilm are densely packed communities of microbial cells that grow on living or inert surfaces and surround themselves with secreted polymers. Many bacterial species form biofilm, and their study has revealed them to be complex and diverse. The structural and physiological complexity of biofilm has led to the idea that they are coordinated and cooperative groups, analogous to multicellular organisms [1].

A biofilm is a population of cells growing on a surface and enclosed within an exopolymer matrix that can restrict the diffusion of substances and bind antimicrobials. This will provide effective resistance for biofilm cells against large molecules such as antimicrobial proteins lysozyme and complement [2]. Biofilm are ubiquitous on surfaces in drinking-water distribution systems, where they generally occur in the form of a thin and heterogeneous surface colonization. However, certain types of elastomeric plumbing materials as well as rubber-coated valves in drinking-water distribution systems have been observed to support substantial biofilm development, which was supposed to be due to the release of biodegradable compounds providing favourable nutrient conditions [3][4][5]. Drinking-water biofilm are formed predominantly by heterotrophic microorganisms of the autochthonous aquatic microflora without any relevance to human health. Occasionally, biofilm can act as a reservoir for microorganisms with pathogenic properties [6][4]. When these organisms persist and multiply within biofilm, and are released from the biofilm into the water phase, they result in the deterioration of the hygienic quality of drinking water and pose a potential threat to human health. The interest in antibiotic susceptibility tests for biofilm bacteria has increased in the last few years [7]. Bacterial biofilm are often associated with long-term persistence of organisms in various environments. Bacteria in biofilm display dramatically increased resistance to antibiotics [8]. Bacteria embedded within biofilm present a challenge to surface decontamination by conventional means [9]. In modern clinical microbiology, establishment of bacterial biofilm is considered a pathogenicity trait during chronic infections [10]. There is a difficulty encountered in the eradication of a chronic infection associated with biofilm formation and this difficulty lies in the fact that biofilm bacteria are able to

- Okafor Collins Onyebuchi Okeke, Department of Applied Microbiology, Faculty of Biological Sciences, Ebonyi State University, P.M.B 053, Abakaliki, Nigeria. E-mail: cofor1@yahoo.com
- Nwadiogbu Ifeanyi Anthony, Department of Applied Microbiology, Faculty of Biological Sciences, Ebonyi State University, P.M.B 053, Abakaliki, Nigeria. E-mail: tonycan553@gmail.com
- Agumah Nnabuife Bernard, Department of Applied Microbiology, Faculty of Biological Sciences, Ebonyi State University, P.M.B 053, Abakaliki, Nigeria. E-mail: buiifewenemighty@gmail.com
- Eni Obehi Eugene, Department of Applied Microbiology, Faculty of Biological Sciences, Ebonyi State University, P.M.B 053, Abakaliki, Nigeria. E-mail: pascejoy@gmail.com
- Odinkemere Stephen Chijindu, Department of Dialysis, Abia State Specialist Hospital & Diagnostic Centre, Umuahia, Nigeria.

resist higher antibiotic concentration than bacteria in suspension [11]. The intracellular biofilm like properties allows bacteria to outlast a strong immune response to establish a dormant reservoir of pathogens inside the bladder cells [12]. The presence of coliform bacteria in the water supply indicates recent contamination by human or animal faeces. Coliform bacteria are group of anaerobic, lactose-fermenting bacteria, of which *Escherichia coli* is the most important member. Most coliform are not harmful, but since they arise from faeces, they are useful as a test of faecal contamination, and particularly as a test for water pollution. Typical genera include *Enterobacter*, *Escherichia*, and *Klebsiella*. [13]. Coliform bacteria are known to be exclusively transmitted through faecal contaminated water and these are responsible for massive epidemics of enteric diseases like cholera, diarrhoea, typhoid fever and dysentery e.t.c [14]. Coliform bacteria are regarded as belonging to the genera *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Haffnia* and *Serratia*. Although coliform organisms may not always be directly related to the presence of faecal contamination, or pathogens in drinking water, the coliform test is still useful for monitoring microbial quality of treated piped borne water supplies [15]. Since water is universally consumed in large quantity, it is important to know the types and number of microbes taken in by drinking water [16]. In developing countries including Nigeria, where the majority of the people live in rural areas, rivers, streams, well and more recently boreholes, serve as the main sources of water for drinking and domestic use) [17]. The underground water supplies are usually consumed safe provided they are properly located, constructed and operated according to the World Health Organization Guidelines for Drinking Water [18]. In developed and developing countries, underground water supply at least 100 million people with drinking water and Nigeria as a developing country depends largely on underground water for most of her domestic works such as washing, cooking and drinking. Globally, underground water provides 25% of its drinking water [19][20]. Water has played a significant role in the transmission of human diseases and indicator organism (i.e. coliform) [21]. Potential health risk may exist due to the microbial content of sachets/table water and borehole waters since water is one of the vehicles for the transmission of pathogenic organisms [22].

MATERIALS AND METHODS

Collection of Water Samples

Five hundred samples of water comprising two hundred and fifty sachet water from different companies (Aqua Rapha, Bejoy, Gospel, Nene, and Rock Tama) and two hundred and fifty borehole samples from bore hole water dispensers Metropolis were collected. They were transported in cool boxes to the Laboratory of the Department of Applied Microbiology, Ebonyi State University, Abakaliki, Nigeria for immediate analysis. Biofilm samples were contemporaneously collected from both borehole and sachet water for biofilm sampling at different drinking water distribution systems. After flushing the borehole nozzles for the borehole samples, samples were taken in sterile bottles containing 10% sodium thiosulphate. Collection was done by scraping the internal surface of the borehole nozzles by

vigorously rubbing the surface with sterile swabs soaked in sterile distilled water. Swab samples were then kept wet in few millilitres of water collected from the borehole nozzles. All the samples were transported under refrigerated conditions into the laboratory and analyzed within the same day of sampling. While for sachet water sample, the water inside the sachet was emptied and the sachet sack was air dried under room temperature after which the sack was swabbed with a sterile swab and inoculated on nutrient agar for identification of coliform bacteria [23].

Microbial Analysis

Five hundred water samples were collected. Fifty samples each were collected from five different batches of sachet waters and different locations of borehole waters. They were analyzed for the presence of coliform bacteria using standard Microbiological techniques. The Multiple Tube Fermentation Technique (MPN) presumptive test which involves adding the water sample to a set of test tubes was used each of which contains a triple strength lactose broth with a bromocresol purple indicator and an inverted Durham tube. The tubes were then incubated at $35 \pm 0.5^\circ\text{C}$ for 24 to 48 hours for total coliform and at $44.5 \pm 0.2^\circ\text{C}$ for faecal coliform. Gas productions were observed in an inverted tube after incubation for the presence of total coliform. The MPN confirmatory test was carried in brilliant green bile lactose broth and MPN completed test using eosinemethylene blue (EMB) agar. Further characterization was carried out by using biochemical reactions viz: indole, methyl red, Voges-Proskauer and citrate utilization test (IMVIC), motility and Gram staining [24]. The test for exopolymer capsule that indicates the existence of biofilm was done using the Maneval's solution method using Congo red as reagent on a glass slide. The slide was examined with an oil immersion lens at 1,000X magnification for a white capsule in red, white, and blue preparation [25][26][27].

Antibiotic susceptibility studies

Sensitivity testing of *E.coli*, *Klebsiella* sp and *Enterobacter* sp isolates to different antibiotics was performed by disc diffusion method. Sterile nutrient agar was prepared and a 0.5MacFarland equivalent standard of the test organisms were streaked with a sterile non cotton swab that was dipped into the standardized inocula on the surface of the agar and allowed to pre-diffuse for 15-20minutes. The following antibiotics discs Nitrofurantoin(100µg), Ciprofloxacin(100µg), Tetracycline(50µg), Norfloxacin / Norbactin (10µg), Amoxycillin(30µg), Ofloxacin(5µg), Chloramphenicol(10 µg), Cefuroxime(30 µg), Ampicillin(30µg), Gentamicin(10µg) were aseptically placed on the surface of the agar plates with a sterile forceps. These were incubated at 35°C for 18-24hours, after which the inhibition zone diameter (IZD) in mm was taken and interpreted using CLSI standard [28].

RESULTS

A total number of 138 (55.2%) bacterial isolates were found in the 250 samples of sachet water brands. *E. coli* was found to be 79(31.6%), *Klebsiella* spp, 30 (12%), *Enterobacter* spp, 23 (9.2%) and *Pseudomonas* spp,6 (2.4%). The contamination in Aqua Rapha was as follows: *E.coli*, 19(38%); *Klebsiella* spp, 9(18%) and *Enterobacter*

spp, 7 (14%); in Bejoy; *E. coli*, 12 (24%); *Klebsiella* spp, 1 (2%); *Enterobacter* spp, 6(2.4%) and *Pseudomonas* spp, 6(2.4%); in Gospel; *E. coli*, 33 (66%); *Klebsiella* spp,

11(22%) and *Enterobacter* spp, 10 (20%). In Nene, *E. coli*, 9(18%); *Klebsiella* spp, 5(10%); while in Rock Tama, *E. coli*, 6 (12%) and *Klebsiella* spp, 4 (8%) (Table 1)

Table 1: Percentage Number of Coliform Bacteria Isolates from Sachet Water Brands

| Sachet water brands | Coliform Isolates/Bacteria | | | | Total |
|---------------------|----------------------------|-----------------------|-------------------------|------------------------|------------|
| | <i>E. coli</i> | <i>Klebsiella</i> Spp | <i>Enterobacter</i> spp | <i>Pseudomonas</i> spp | |
| Aqua Rapha | 19 | 9 | 7 | Nil | 35 |
| Bejoy | 12 | 1 | 6 | 6 | 25 |
| Gospel | 33 | 11 | 10 | Nil | 54 |
| Nene | 9 | 5 | Nil | Nil | 14 |
| Rock Tama | 6 | 4 | Nil | Nil | 10 |
| Total(%) | 79(31.6%) | 30(12%) | 23(9.2%) | 6(2.4%) | 138(55.2%) |

Of the 250 bore-hole water samples, a total number of 173 (69.2%) bacterial isolates were found. *E. coli*, were 80 (32%); *Klebsiella* spp, 47(18.8%) and *Enterobacter* spp, 46 (18.4%). In Abofia the distribution is as follows *E. coli*, 18(50%); *Klebsiella* spp, 10 (27.8%) and *Enterobacter* spp, 12 (33.3%). In Azugwu, the distribution was as follows: *E. coli*, 4 (11.1%); *Klebsiella* spp, 7 (19.4%). In Azuiyokwu, *E. coli*, 6 (16.7%); *Klebsiella* spp, 8 (22.2%) and *Enterobacter* spp, 2 (5.6%). In Azuiyudene, the distribution was as

follows: *E. coli*, 12 (33.3%); *Klebsiella* spp, 4 (11.1%) and *Enterobacter* spp, 7 (19.4%). In Kpirikpiri, the distribution was as follows: *E. coli*, 24 (66.7%); *Klebsiella* spp, 6 (16.7%) and *Enterobacter* spp, 10 (27.8%). In Presco/Ntezi the distribution is as follows: *E. coli*, 9(25.7%); *Klebsiella* spp, 6(17.1%) and *Enterobacter* spp, 10 (28.6%). in Udensi, the distribution of the faecal contamination is as follows: *E. coli*, 7 (20%); *Klebsiella* spp, 6 (17.1%) and *Enterobacter* spp, 5 (14.3%). (Table 2)

Table 2: Number of Coliform Bacteria Isolates from Borehole Water Samples

| Samples/Locations | Coliform Isolates | | | Total |
|-------------------|-------------------|-----------------------|-------------------------|---------------|
| | <i>E. coli</i> | <i>Klebsiella</i> spp | <i>Enterobacter</i> spp | |
| Abofia | 18 | 10 | 12 | 40 |
| Azugwu | 4 | 7 | Nil | 11 |
| Azuiyokwu | 6 | 8 | 2 | 16 |
| Azuiyudene | 12 | 4 | 7 | 23 |
| Kpirikpiri | 24 | 6 | 10 | 40 |
| Presco/Ntezi | 9 | 6 | 10 | 25 |
| Udensi | 7 | 6 | 5 | 18 |
| Total(%) | 80 32% | 47 18.8% | 46 18.4% | 173 69.32% |

Of the 30 samples of biofilm (i.e. 20 samples for sachet water brand and 10 samples for bore-hole waters), 1(25%) *E. coli* was found in Aqua Rapha, 3(50%) in Bejoy, 3(50%) in Gospel, 2(50%) in Rock Tama and 3(30%) in bore-hole samples while there was no bacteria isolated from the biofilm of Nene water brand. For *Klebsiella* spp; 2(33.3%) was isolated from Bejoy, 6(60%) from bore-hole samples

while there was absence of the organism in Aqua Rapha, Gospel, Nene and Rock Tama water brands. For *Enterobacter* spp 1 (25%) was isolated from the biofilm of Aqua Rapha, 1(16.7%) in Bejoy, 4(40%) from bore-hole samples, while there no organism found in Gospel, Nene, and Rock Tama water brands (Table 3).

Table 3: Percentage of Coliform Bacteria from Biofilm of Sachet Water Brands and Borehole Waters

| Bacteria names | Names of Sachet Water /Borehole samples | | | | | |
|-------------------------|---|-----------|----------|---------|-----------|------------|
| | Aqua Rapha | Bejoy | Gospel | Nene | Rock Tama | Bore-holes |
| <i>E. coli</i> | 1(25%) | 3(50%) | 3(50%) | 0(%) | 2(50%) | 3(30%) |
| <i>Klebsiella</i> spp | Nil(0%) | 2(33.33%) | Nil (0%) | (0%)Nil | 0%(Nil) | 6(60%) |
| <i>Enterobacter</i> spp | 1(25%) | 1(16.67%) | Nil(0%) | (0%)Nil | Nil (0%) | 4(40%) |

The bacteria isolated from biofilm of sachet water and borehole waters were susceptible to three of the antibiotics used. For nitrofurantoin *E. coli*, *Klebsiella* spp and *Enterobacter* spp showed a susceptibility pattern of 8.4%, 100%, and 100% respectively. For amoxicillin, *E. coli* showed a (90%) susceptibility, *Klebsiella* spp (25%) and *Enterobacter* spp (50%). *E. coli*, *Klebsiella* spp, and *Enterobacter* spp also displayed a susceptibility pattern for ampicillin, as follows *E. coli* (16.7%), *Klebsiella* spp (33.4%)

and *Enterobacter* spp (50%). The coliform isolates were completely resistant to the following antibiotics ciprofloxacin, tetracycline, norbactin/norfloxacin, ofloxacin, cefuroxime and gentamicin (Table 4). This showed that they exhibit multi-drug resistance pattern which is a common feature of medically important biofilm bacteria.

Table 4: Percentage Resistance of Coliform Bacteria from Biofilm to Different Antibiotics

| Bacteria Isolates | Antibiotics/Disc conc (Ug/ml) | | | | | | | | | |
|------------------------|-------------------------------|-------------|------------|------------|------------|-----------|-----------|------------|------------|-----------|
| | N (100) | CIP (95) | TE (50) | NB (10) | AX (30) | OF (5) | C (10) | CF (30) | AM (30) | G (10) |
| <i>E. coli</i> | 91.6% | 100% | 100% | 100% | 10% | 100% | 100% | 100% | 83.3% | 100% |
| <i>Klebsiellasp</i> | 0% | 100% | 100% | 100% | 75% | 100% | 100% | 100% | 66.6% | 100% |
| <i>Enterobacterspp</i> | 0% | 100% | 100% | 100% | 50% | 100% | 100% | 100% | 50% | 100% |

CF = Cefuroxime, AM = Ampicillin, G = Gentamicin, N = Nitrofurantoin, CIP = Ciprofloxacin, TE = Tetracycline, NB = Norbactin (Norfloxacin), AX = Amoxycillin, OF = Orfloxacin, C = Chloramphenicol

Results revealed that faecal contamination was high in bore-hole water samples than in sachet water samples. This may be as result of the fact that sachet undergo treatment while almost, if not all borehole waters do not undergo any kind of treatment before they are dispensed to the public for consumption. The bacterial isolates from both sachet water brands and bore-hole waters showed high resistance pattern to different antibiotics used in-vitro, but differed significantly from those isolated from the water biofilm. Results also revealed a high faecal contamination levels in Gospel, Aqua Rapha and Bejoy among the sachet water brands examined, with Nene and Rock Tama sachet water brands having the lowest contamination level.

DISCUSSION

The bacteriological quality of sachet-packed water and borehole waters offered for sale in Abakaliki metropolis was investigated in this study. The results of findings revealed that the popular products that are sold to the public under a wide variety of brand names and borehole waters were unsafe for human consumption except for the sachet packed water with the brand name Rock Tama and Nene that showed low level of contamination. This suggests that the sanitary procedures in the production lines of these commercially sold products did not meet the World Health Organization and the United States Environmental Protection Agency standard for faecal coliform in drinking water which is zero faecal coliform per 100ml [18][29][30]. The borehole water sold in Abakaliki Metropolis for human consumption showed high level of contamination with faecal coliform. Among all the sachet water brands, Rock-Tama and Nene showed a very minimal level of contamination with both *E.coli* and other isolates. This study supports the result of other researchers in the case of faecal contaminations in sachet water brands produced in the South Eastern States of Nigeria and bacteriological analysis of bore-hole waters in some parts of South Eastern Nigeria [31][32][17]. Epidemiological investigations have proved that diarrhea has a world-wide occurrence and accounts for 4% deaths and 5% of health loss to disability. It was also recorded that there are about 4 billion cases of diarrhea on a yearly basis [33]. Bacteria especially the family Enterobacteriaceae which are agents of water-related diarrhea are very different and significant in checking the microbiological quality of water meant for human use, as they are potentially present in contaminated water. Since an outbreak of disease from drinking water has been reported world-wide and that it has been estimated that water-borne disease such as diarrhoeal diseases, schistosomiasis, trachoma, dysentery, ascariasis, typhoid diseases might

account for one-third of the intestinal infections globally [33][34][31]. *Escherichia coli* has been a central organism in water microbiology for decades as an indicator of faecal pollution, and its role as a pathogen rather than an indicator, in drinking water is been stressed in recent studies. This interest in the role of *E.coli* as a cause of diarrhoeal disease has increased because of the emergence of *E. coli* 0157:H7 and other enterohaemorrhagic *E. coli* due to the severity of the related disease [35]. From the above report according to Paul [35], it is obvious that the presence of *E. coli* in sachet water brands and borehole waters sold in Abakaliki metropolis could pose a serious threat to public health. The high faecal coliform content observed in the water samples analysed was indicative of the likely presence of other pathogenic organisms such as *Aeromonas* spp or *Pseudomonas* spp. For instance, about 6(2.4%) isolates of *Pseudomonas* spp were identified in Bejoy sachet water product, and this may pose a health risk in the consumption of the water and its use for domestic purposes. The biofilm isolates showed a high resistance pattern with 70% resistance to the antibiotics used. This shows that their resistance to commonly used antibiotic was very high which could be as a result of the ability of biofilm to facilitate the adherence of these microorganisms to biomedical surfaces and protect them from host immune system and antimicrobial therapy [36]. This view was in agreement with the work of Lewis [37], who reported that according to a recent public statement from the National Institutes of Health in America; more than 65% of all microbial infections are caused by biofilm. This high resistance to antibiotics is due to the formation of cell in the biofilm network called persists [38]. Their susceptibility to only 30% of the antibiotics used could reveal the persistence of gastrointestinal infections and repeated occurrence of diarrhoeal diseases as observed globally. This is because of the role of biofilm in protecting enterovirulent *E. coli* and this poses a serious threat to human health.

CONCLUSION/RECOMMENDATION

The presence of faecal organisms in treated water for human consumption should pose a significant health concern and quality improvement to health workers such as National Food and Drug Administration Control (NAFDAC), and the manufacturers as to make necessary investigation to identify the point of entry and get it rectified as the public health importance of safe drinking water is inevitable. The high load of faecal coliform in drinking water calls for a reassessment of water treatment methods especially for borehole and sachet packed waters since they are the major sources of water used for domestic purposes and drinking.

This study suggests that more check should be put in place in order to maintain the WHO standards for borehole water drilling which recommends that boreholes should be located at least 30m away from latrines and 17m from septic tanks as most of the faecal contamination of pipe waters is caused by the seepage of faecal materials into leaking pipes since most sachet water products are processed from borehole waters. Checks are supposed to be put in place also for the sachet waters by ensuring that the sanitary standard of its production is of high quality. Also government should put more effort in establishing technologies that will enhance a greater understanding of biofilm producing E .coli in recurrent gastrointestinal tract and urinary tract infections which will help in the development of new and more effective treatment of these problematic diseases. There is need to develop cheap and effective small scale water disinfection methods for making borehole water safe for domestic purposes thus constant washing of storage tanks which enhances appropriateness of water storage, solar disinfection, boiling and chlorination as points of small scale method for making borehole water fit for domestic usage.

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